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STUDIES OF OIL FROM COWPEA (*VIGNA UNGUICULATA* (L.) WALP.) CULTIVARS COMMONLY GROWN IN PAKISTAN

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Abstract

The physiochemical properties and fatty acid (FA) composition of oil from seeds of 4 cowpea cultivars viz., Elite, CP_1, White Star and SA dandy, commonly grown in Pakistan, were investigated. Oil contents ranged from 2.71-2.96% with triacylglycerols being present in highest amount. Iodine values were found highest in CP1 while SA dandy and CP1 excelled in saponification values and acid values, respectively. Despite variations unsaturated fatty acids were observed as being present in higher concentration in all cultivars. Among sterols, stigmasterol was present in highest amount followed by β-sitosterol and campesterol. Among tocopherols, α-, and β-tocopherols were observed as being present in highest and lowest concentrations, respectively. Results from most of the parameters revealed not significant ($p \leq 0.05$) differences among the cultivars. The results showed that campestrol positively correlated with stigmasterol, Δ^5-avenasterol, Δ^7-avenasterol, and have high negative (-0.9) correlation with β-sitosterol. However, campesterol (sterol) has only negative correlation with *α*-tocopherol, while have positive correlations with *β*-tocopherol, *γ*-tocopherol and *δ*-tocopherol. But stigmasterol has negative correlations with *α*-tocopherol (-0.7; high), *β*-tocopherol (-0.3, low) and δ-tocopherol (-0.2, low) while have low positive (+0.4) with *γ*-tocopherol. β-sitosterol also depicted similar correlations with different tocopherols. All cowpea cultivars appeared to be suitable as nutritional oil source of comparable quality.

Introduction

Legumes including cowpea have been widely grown in Pakistan and their seeds are used as human and animal food to provide calories and protein. As food, cowpea seeds are eaten in different forms; they could be boiled, parched, fried, roasted, mixed with sauce or stewed and consumed directly. Its seeds are consumed in different forms as they provide important vitamins, phyto-nutrients including antioxidants besides carbohydrates, minerals and trace elements. In addition, it is a cheap source of high quality protein in the diets of millions in developing countries like Pakistan, who cannot afford costly animal protein for balanced nutrition (Singh *et al.,* 1997; Moses, 2006).

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Cowpea is used medicinally by grinding the seed mixed with oil to treat stubborn boils (Duke, 1990). The cooking liquor of the seeds with spices is considered to be a potential remedy for the common cold (Siddhuraju & Becker, 2007). Cowpea starch jelly is used against thirst (Gao, 1989). The leaves and seeds are applied as a poultice to treat swellings and infections, leaves are chewed to treat tooth ailments, powdered carbonized seeds are applied on insect stings, the root is used as an antidote for snakebites and to treat epilepsy, chest pain, constipation, and dysmenorrheal and unspecified plant parts are used as a sedative in tachycardia and against various pains (Brink & Belay, 2006). The seed is diuretic. It is used to strengthen the stomach. When boiled and eaten as a food it is considered to destroy worms in the stomach (Chopra *et al*., 1986). An infusion of seed can be taken orally to treat amenorrhea whilst powdered roots eaten with porridge are believed to treat painful menstruation, epilepsy and chest pain (Van Wyk & Gericke, 2000). Leaves are applied on burns and can be used as a snuff to treat headaches. Emetics made from the plant are taken to relieve fever (Hutchings *et al*., 1996). Traditional healers use it to treat urinary shistomiasis (bilharzias) (Ndamba *et al*., 1994). The seeds are cooked with the roots of other herbs to treat blood in urine and bilharzias (Nyazema, 1987; Kritzinger *et al*., 2004).

Cowpea seed can be also crushed to obtain edible oil although it cannot be described as an oil-bearing seed. The oil content of cowpea, grown in the different parts of world, reportedly is relatively low on an average (2.48%-3.03%) (Mahadevappa & Piyara, 1978, 1981; Onwuliri & Obu, 2002). Tocopherols are the most important lipophilic antioxidants. They protect polyunsaturated fatty acids in cell membranes and lipoproteins from oxidation. In addition, they exhibit a number of other biological activities, including effects on cellular signaling and the prevention of infertility in animals, and are believed to play a preventive role in diseases associated with oxidative stress like cancer, cardiovascular diseases, cataracts, age-related macular degeneration, central neurodegenerative diseases and diabetes mellitus (Brigelius-Flohe *et al*., 2002).

As the commercial interest is growing, chemical studies are necessary to elucidate the phytochemical analysis of different legume seed oils. Cowpea is the major legume crop in some areas of Pakistan and recently its production has increased substantially. To our knowledge, no data has been reported on the characterization and compositional studies of the oil obtained from the seeds of the cowpea cultivars grown in Pakistan. In this context, as part of our continuous studies on exploring the hidden potential of indigenous flora of Pakistan and analysis of oil of legume crops (Zia-ul-Haq *et al*., 2007 a, b; 2008 a, b; 2009, Ahmad *et al.,* 2009 a, b; Shad *et al.,* 2009; Nisar *et al*., 2010) we have analyzed oil of cowpea seed. This study will provide a database for this crop which has not been so extensively explored so far.

Materials and Methods

The seeds of 4 cowpea (*Vigna unguiculata* (L.) Walp.) cultivars viz., Elite, CP1, White Star and SA dandy were procured from the Department of Agronomy, Bahauddin Zakariya University, Multan, Pakistan. After removing immature and damaged seeds, seeds of all the cultivars were divided into groups for storage in stainless-steel containers at 4°C before analyses. The solvents (Fisher Scientific, Loughborough, UK) used were of analytical grade and were not further purified.

Extraction of seed oil: The seeds were ground to flour with an IKA all basic mill (IKA Works Inc., Wilmington, NC, USA) and were passed through a 60-mesh sieve. The seed powder was extracted with a mixture of n-hexane/2-propanol (3:1, V/V) in a Soxhlet apparatus (6 h).

General properties and oil composition: Determination of iodine value (IV), acid value, and saponification value (SV), of the extracted oil was carried out by standard IUPAC methods 2.205, 2.201, and 2.504, respectively for the analysis of fats and oils (Anon., 1987). Qualitative analysis of oil was accomplished by a reported method (Malins & Mangold, 1960) using silica gel, G 60 Merck type 5721 and 20 cm 9 20 cm glass plates with 0.25 mm thickness. The developing solvent system was n-hexane: diethyl ether: acetic acid glacial (80: 20: 2, V/V/V). The separated fractions were visualized by exposure to iodine vapor in a closed chamber after drying. All fractions were identified on thin layer plates by comparing their Rf values with those of known standards. For quantitative analysis, the different lipid fractions were scanned by using Shimadzu TLC-Scanner (C-S-910).The area under each peak was measured by the triangulation method (Kates, 1972). The percentage of each component was calculated with regard to the total area as follows by a reported procedure (El-Sayed *et al*., 2007).

$$\%\,\text{Component} = \left(\frac{Area\,of\,each\,peak}{Total\,peaks\,area} \right) \times 100$$

Fatty acid composition: Fatty acid methyl esters (FAMEs) were prepared according to the standard of IUPAC method 2.301 (Anon., 1987) and analyzed on a SHIMADZU gas chromatograph model 17-A with flame ionization detector (FID). Separation was done on a capillary column SP 2330 (30 m x 0.32 mm x 0.25 μm; Supelco; Bellefonte, Pa., U.S.A.). Nitrogen was used as a carrier gas at a flow rate of 3.0 mL/min. Column temperature was programmed from 180 to 220°C @ 3°C/min. Initial and final temperatures were held for 2 and 10 min, respectively. Injector and detector were kept at 230 and 250°C, respectively. A sample volume of 1.0 μL was injected with the split ratio of 1:75. FAMEs were identified by comparing their relative and absolute retention times to those of authentic standards. The quantification was done by a Chromatography Station for Windows (CSW32) data handling software (Data Apex Ltd. CZ-158 00 Pague 5, the Czech Republic). The fatty acid composition was reported as a relative percentage of the total peak area and the results were calculated as mg/100 g of dry matter (Table 3).

Sterol composition: The determination of sterols was made following the official method of the Association of Official Analytical Chemists (Anon., 1984). Analysis was carried out on a Perkin Elmer gas chromatograph model 8700, equipped with methylphenyl polysiloxane coated capillary column OV-17 (30 m × 0.25 mm, 0.20 μm film thickness) and a flame ionization detector (FID). The column was isothermally operated at temperature of 255°C. Injector and FID temperature were set at 275 and 290°C, respectively. Extra pure N_2 at a flow rate of 3 mL/ min was used as a carrier gas. The internal standard used was 5-α-cholestane. Identification and quantification of unknown sterol components was made using a pure sterol standard mixture (Table 4).

Table 1. Oil contents and physical properties of oil from cowpea cultivars.

Cultivar	Oil (%)	Saponification number (mg KOH/g oil)	Acid value (mg KOH/g oil)	Iodine (g/100 g oil)	Refractive index (40 ^{0}C)	Unsaponifiable matter (%)
CP1	2.71±0.52a	177±0.04ab	1.60±0.11a	117.0±0.1a	1.48±0.04a	2.36±0.28c
Elite	2.96±0.05a	175±0.01b	1.40±0.29b	110.0±0.3ab	1.48±0.06a	2.81±0.07a
White star	2.86±0.05a	179±0.02ab	1.30±0.07ab	102.0b±0.4b	1.47±0.09a	2.49±0.21bc
SA dandy	2.77±0.03a	180±0.01a	1.50±0.06a	113.0±0.2ab	1.49±0.01a	2.63±0.24ab

Data are expressed as means ± standard deviations *(n* = 3), values marked by the same letter in same column of same class are not significantly different *(p*<0.05).

Table 2. Percentage composition of lipid classes in oils from seeds of cowpea cultivars.

Lipid fraction (%)	Elite	CP1	White star	SA dandy
Phospholipids	25.02	24.52	24.33	25.66
Monoglycerides	10.08	10.19	11.04	10.81
Diglycerides	7.20	8.17	7.45	7.99
Sterols	6.80	5.68	5.04	5.23
Free fatty acids	7.13	8.27	7.90	7.58
Triglycerides	40.77	41.01	42.17	40.14
Hydrocarbons + sterol esters	3.00	2.16	2.07	2.59

Table 3. Fatty acid composition (% w/w) of oil from cowpea cultivars.

Fatty acid	Elite	CP1	White star	SA dandy
16:0	20.57 ± 0.03ns	19.85 ± 0.05	19.21 ± 0.09	19.98 ± 0.07
16:1	4.16 ± 0.02ns	3.93 ± 0.04	3.77 ± 0.03	3.56 ± 0.09
18:0	5.04 ± 0.07a	6.02 ± 0.08b	6.99 ± 0.05a	5.72 ± 0.01c
18:1	15.25 ± 0.08ns	16.01 ± 0.05	14.97 ± 0.08	15.82 ± 0.07
18:2	34.77 ± 0.05ns	35.21 ± 0.03	36.01 ± 0.05	33.89 ± 0.05
18:3	20.21 ± 0.07ns	18.98 ± 0.2	19.05 ± 0.01	21.03 ± 0.06

Means ± standard deviations *(n* = 3), means marked by the different letter in same row are significantly different *(p*<0.05).

Table 4. Sterol composition (%) of oil from cowpea cultivars.

Sterols	CP1	Elite	White star	SA dandy
Campesterol	10.1 ± 0.43ns	13.3 ± 0.29	12.5 ± 0.49	13.4 ± 0.45
Stigmasterol	41.4 ± 0.25ns	43.2 ± 0.19	42.1 ± 0.24	40.8 ± 0.08
β-sitosterol	33.8 ± 0.11ns	28.9 ± 0.06	30.2 ± 0.07	29.1 ± 0.16
Δ^5-avenasterol	13.0 ± 0.09ns	12.7 ± 0.25	13.5 ±002	14.3 ± 0.14
Δ^7-avenasterol	1.7 ± 0.04b	1.9 ± 0.06b	1.7 ± 0.09c	2.4 ± 0.09a

Means ± standard deviations *(n* = 3), means marked by the different letter in same row are significantly different *(p*<0.05).

Tocopherol contents: The tocopherol isomer determination was carried out by HPLC according to a reported method (Sierra *et al*., 1996). Briefly a modular chromatograph system (Water Associates, Mildford, CT, USA), equipped with a model 510 pump, a Rheodyne 7000 sample injector and a Waters 470 scanning fluorescence detector at λ_{ex} 295 and λ_{em} 330 nm. A 90:10 mixture of *n*-hexane: diisopropyl ether (Scharlau, HPLC grade) was used as mobile phase at a flow of 1.2 ml/min. A Lichrosorb Si 60 (250 × 46 mm i.d., 5 µm) column (Technokroma) connected to a guard column Phenyl/Corasil Bondapak (40 × 46 mm i.d.) and a 50 µl loop were used. Data were processed on a PC (NEC Corporation, Boxborough, MA) with a Maxima Database (Millipore Corporation, Waters Chromatography division, Melford, MA, USA) Table 5.

Table 5. Tocopherol contents (mg/100 g) of cowpea cultivars.

Cowpea cultivar	*α*-tocopherol	*β*-tocopherol	*γ*-tocopherol	*δ*-tocopherol
CP1	3.09 ± 0.21ns	0.03 ± 0.09d	0.41 ± 0.40ns	1.75 ± 0.23ns
Elite	2.95 ± 0.39	0.05 ± 0.31c	0.46 ± 0.34	1.77 ± 0.28
White star	2.91 ± 0.17	0.07 ± 0.20b	0.39 ± 0.11	1.86 ±0.14
SA dandy	3.07 ± 0.04	0.09 ± 0.09a	0.43 ± 0.07	1.83 ± 0.02

Means ± standard deviations *(n* = 3), means marked by the different letter in same column differ significantly different *(p*<0.05).

Statistics analysis: Data analysis was carried out using the analysis of variance function of the "MSTATC" statistical computer package (Anon., 1991). Differences among the means were determined using the LSD test ($p<0.05$) when more than two means were found to be significant (Steel *et al*., 1997).

Results and Discussion

The exploration of newer dietary sources of bioactive compounds, having profound health benefits given by fatty acids, sterols and tocopherols, is focus of research among food scientists. From the results it is clear that seed oil content ranged from 2.7 3.0%. Moreover, cultivars of cowpea did not differ in seed oil content though small and non-significant differences were obtained. The low oil yields from different cowpea cultivars can be supported with the fact that legumes are generally not oil bearing crops (Mabaleha & Yeboah, 2004). The physicochemical constants of the oils indicate quality of seed oil. The saponification numbers seed oil of all cowpea cultivars were lower than 200 with low acid values and high iodine numbers. These results suggest that seed oil of all cowpea cultivars have higher oxidative stability and protection during storage and processing. These results are similar with those of Zia-ul-Haq *et al*., (2007; 2008) who found approximately similar profile in seed oil of different leguminous crops such as *Cicer arietinum* and *Vigna radiata*.

Oil constituents of cowpea cultivars (Table 2) show that cowpea cultivars did not differ significantly in composition of seed oil. Of mono-, di and tri-aceyl glycerols, triacyl glycerol was the highest in seed oil of all cowpea cultivars which is similar to some earlier studies on cowpea from other countries (Mahadevappa & Piyara, 1978; Onwuliri & Obu; 2002; Omogbai, 1990) and other legumes (Zia-ul-haq *et al*., 2007; 2008; 2009). Moreover, the quantity of free fatty acids in seed oil of all cultivars are very low indicating that seed oil from all cowpea cultivars is of high quality. Fatty acid profile of all cowpea cultivars reveals the lipids as a good source of saturated and unsaturated fatty acids as palmitic and stearic acids. The presence of high levels of unsaturated fatty acids, in all the presently studied cultivars, is nutritionally desirable and results are comparable to previously published works (Adebooye & Singh, 2007). Saturated fatty acids contributed little of the total fatty acids content (Table 3). Linoleic and linolenic acids are the most important essential fatty acids required for growth, physiological functions and maintenance in animals (Pugalenthi *et al*., 2004). In particular, the nutritional value of linoleic acid is due to its metabolism at tissue levels which produce the hormone-like prostaglandins which activity includes lowering of blood pressure and constriction of smooth muscle (Aurand *et al*., 1987). Fatty acid contents are in partial agreement with that reported earlier for other legumes (Zia-ul-haq *et al*., 2007; 2008).

Plant sterols possess a broad spectrum of therapeutic effects in animals and humans. In humans, consumption of plant-derived sterols, particularly β-sitosterol, reduces blood pressure serum cholesterol levels, and the risk of chronic heart diseases (Ling & Jones,

1995; Moreau *et al*., 2002; Clark, 1996). Furthermore results for sterol profile in all cowpea cultivars (Table 4), it is clear that appreciable contents of campesterol, stigmasterol and avenasterol were recorded. Sitosterol was the principal sterol in all of the four investigated cowpea cultivars which is in line with reported literature for low-oil beaing legumes like mungbean and chickpea in general (Zia-ul-haq *et al*., 2007; 2008; 2009) and for cowpea in particular (Gaydou *et al*., 1983). Most of the plants belonging to family *Leguminosae* so far examined are reported to contain campestral, sitosterol and stigma sterols as the dominant sterols (Akihisa *et al*., 2000). Regional and cultivars variations for the distribution of campesterol, stigmasterol, β-sitosterol, Δ^5, avenasterol and clerosterol in oils and fats have already been reported in the literature (Norman, 1979; Rossell, 1991). Phytosterols also serve as intermediates for the synthesis of hormonal sterols and other related pharmaceuticals (Clark, 1996). Furthermore, phytosterols, especially, β-sitosterol, exhibit significant anti-inflammatory effects and antitumor properties (Ling & Jones, 1995; Moreau *et al*., 2002). In addition, phytosterols are known as antipolymerization factors and as antioxidants, especially those containing an ethylidene group in the aliphatic side chain (Δ^7- and Δ^5-avenasterols), in vegetable oil at frying temperature (Wang *et al*., 2002).

Of various tocopherols (Table 5) *β*-tocopherol was observed in lowest concentration in all cultivars while appreciable contents of *δ*-tocopherol followed *γ*-tocopherol were also observed. Although all cowpea cultivars examined in the present study contained all major tocopherols, the tocopherol contents are relatively low as has been observed earlier in other legumes (Zia-ul-haq *et al*., 2007; 2008; 2009) and for cowpea in particular (Doblado *et al*., 2005; Ching & Mohamed, 2001). It is already known that α-Tocopherol, is most common form of vitamin E present in nature, is the most biologically active (Bjorneboe *et al*., 1990). It is preferentially retained in large quantities and transported to body components (Traber *et al*., 1990). Of cowpea cultivars examined in the present study, cv. CP1 was maximal in *α*-tocopherol.

Table 6 depicted the correlation coefficients that describe the level of association among different sterols at 5% of probability level. The results (Table 6) showed that campestrol positively correlated with stigmasterol, Δ^5- avenasterol, Δ^7-avenasterol, and have high negative (-0.9) correlation with β-sitosterol. While, stigmasterol has negative correlation with other sterols, i.e., β-sitosterol, Δ^5- avenasterol, Δ^7-avenasterol except one campestrol in our study. Similarly, β-sitosterol has negative correlation with Δ^5-avenasterol, Δ^7-avenasterol. But Δ^5- avenasterol and Δ^7-avenasterol have high positive (+0.7) correlation with each others. These are in line with the findings with *Cicer arietinum* L., as test crop (Zia-ul-haq *et al*., 2009).

Table 7 shows the correlation coefficients between the sterols and tocopheros at 5% of probability level. Campesterol (sterol) has only negative correlation with *α*-tocopherol, while have positive correlations with *β*-tocopherol, *γ*-tocopherol and *δ*-tocopherol. But stigmasterol has negative correlations with *α*-tocopherol (-0.7; high), *β*-tocopherol (-0.3, low) and δ-tocopherol (-0.2, low) while have low positive (+0.4) with *γ*-tocopherol. β-sitosterol also depicted similar correlations with different tocopherols. Δ^5- avenasterol showed positive correlations with *α*-tocopherol, *β*-tocopherol, and δ-tocopherols, while only negative correlation with the *γ*-tocopherol (Table 7). Unlike, other sterols, the Δ^7-avenasterol, exhibited positive (low to high) correlation with the tocopherols (*α, β, γ,* and δ). Similar correlation coefficient figures were observed by other workers (Zia-ul-haq *et al*., 2009) for another leguminous chickpea (*Cicer arietinum* L.) crop. These correlation coefficients have high importance for the crop breeders, while keeping in view the quality traits of crops, in their breeding programs.

Table 6. Correlation coefficients between sterols from cowpea cultivars.

Characteristics Sterols-Sterols	Correlation coefficient	Characteristics Sterols-Sterols	Correlation coefficient
Campesterol and Stigmasterol	+0.3*	Stigmasterol and Δ^5- avenasterol	-0.7**
Campesterol and β-sitosterol	-0.9***	Stigmasterol and Δ^7-avenasterol	-0.4**
Campesterol and Δ^5- avenasterol	+0.4**	β-sitosterol and Δ^5- avenasterol	-0.3*
Campesterol and Δ^7-avenasterol	+0.6**	β-sitosterol and Δ^7-avenasterol	-0.5**
Stigmasterol and β-sitosterol	-0.3*	Δ^5- avenasterol and Δ^7-avenasterol	0.7**

*=Significant
**= Highly significant
***=Very highly significant

Table 7. Correlation coefficients between sterols and tocopherols from cowpea cultivars.

Characteristics Sterols-Tocopherols	Correlation coefficient	Characteristics Sterols-Tocopherols	Correlation coefficient
Campesterol and *α*-tocopherol	-0.4**	β-sitosterol and *γ*-tocopherol	-0.5**
Campesterol and *β*-tocopherol	+0.7***	β-sitosterol and *δ*-tocopherol	-0.5**
Campesterol and *γ*-tocopherol	+0.4**	Δ^5- avenasterol and *α*-tocopherol	+0.2*
Campesterol and *δ*-tocopherol	+0.5**	Δ^5- avenasterol and *β*-tocopherol	+0.8***
Stigmasterol and *α*-tocopherol	-0.7***	Δ^5- avenasterol and *γ*-tocopherol	-0.2*
Stigmasterol and *β*-tocopherol	-0.3*	Δ^5- avenasterol and *δ*-tocopherol	+0.6**
Stigmasterol and *γ*-tocopherol	+0.4**	Δ^7-avenasterol and *α*-tocopherol	+0.3*
Stigmasterol and *δ*-tocopherol	-0.2*	Δ^7-avenasterol and *β*-tocopherol	+0.7***
β-sitosterol and *α*-tocopherol	+0.5**	Δ^7-avenasterol and *γ*-tocopherol	+0.4**
β-sitosterol and *β*-tocopherol	-0.7***	Δ^7-avenasterol and *δ*-tocopherol	+0.2*

*=Significant
**= Highly significant
***=Very highly significant

From the results of the present study and above-mentioned discussion, it is obvious that quality of seed oil from cowpea cultivars is comparable to the other leguminous crops as cowpea seeds. Therefore, consumption of cowpea seeds as human diet or use in some commercial products providing such nutrients as proteins, carbohydrates, and minerals, may help in widely acclaimed health benefits of the these oil constituents.

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